

Dilutions of the fusion protein test material (50 μ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to
5 a final concentration of 10 μ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 μ C of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the fusion protein of interest are screened in separate experiments and
10 compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

Example 43: Assays for Protease Activity

15 The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacrylamide/0.1% SDS gels containing 1% gelatin
20 or casein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis appear as clear areas against the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mMNaPO₄, 1mM
25 EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are
30 performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

Example 44: Identifying Serine Protease Substrate Specificity

35 Methods known in the art or described herein may be used to determine the substrate

specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

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Example 45: Ligand Binding Assays

The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

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Example 46: Functional Assay in *Xenopus* Oocytes

Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus* oocytes in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca²⁺ free Barth's medium at room temperature. The *Xenopus* system can be used to screen known ligands and tissue/cell extracts for activating ligands.

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Example 47: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus

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capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

Example 48: Extract/Cell Supernatant Screening

5 A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts
10 to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

15 *Example 49: ATP-binding assay*

The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein
20 incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenylyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-
25 azido-ATP (^{32}P -ATP) (5 mCi/ μmol , ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to
30 SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenylyl-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

35 *Example 50: Phosphorylation Assay*

In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein

incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P -ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion protein of the invention is incubated with the protein substrate, ^{32}P -ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P -ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

Example 51: Detection of Phosphorylation Activity (Activation) of an Albumin Fusion Protein of the Invention in the Presence of Polypeptide Ligands

Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

Example 52: Identification Of Signal Transduction Proteins That Interact With An albumin fusion protein Of The Present Invention

Albumin fusion proteins of the invention may serve as research tools for the identification, characterization and purification of signal transduction pathway proteins or receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 53: IL-6 Bioassay

A variety of assays are known in the art for testing the proliferative effects of an albumin fusion protein of the invention. For example, one such assay is the IL-6 Bioassay as described by Marz *et al.* (*Proc. Natl. Acad. Sci., U.S.A.*, 95:3251-56 (1998), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls

containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μ l, and 50 μ l of fusion protein of the invention is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention) relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

Example 54: Support of Chicken Embryo Neuron Survival

To test whether sympathetic neuronal cell viability is supported by an albumin fusion protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A.*, 96:11458-63 (1998), which is herein incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO₂ in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mosmann, T., *J. Immunol. Methods*, 65:55-63 (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

Example 55: Assay for Phosphatase Activity

The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [³²P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from ³²P-labeled MyBP.

Example 56: Interaction of Serine/Threonine Phosphatases with other Proteins

Fusion protein of the invention having serine/threonine phosphatase activity (e.g., as determined in Example 55) are useful, for example, as research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein-complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 57: Assaying for Heparanase Activity

There are numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells (1×10^6 cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S -labeled ECM or soluble ECM-derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

Example 58: Immobilization of biomolecules

This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constructs (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation

upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO₄ and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl₂, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

Example 59: Assays for Metalloproteinase Activity

Metalloproteinases are peptide hydrolases which use metal ions, such as Zn²⁺, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

Proteolysis of alpha-2-macroglobulin

To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl₂, 25 μM ZnCl₂ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases

Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND HgCl₂), peptide metalloproteinase inhibitors (TIMP-1 and TIMP-2), and commercial small molecule MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC₅₀ = 1.0 μM against MMP-1 and MMP-8; IC₅₀ = 30 μM against MMP-9; IC₅₀ = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC₅₀ = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50μg/ml) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M

NaCl, 10 mM CaCl₂, 25 μM ZnCl₂ and 0.05% Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1 μl of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

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Synthetic Fluorogenic Peptide Substrates Cleavage Assay

The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques known in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor-α (TNF-α) converting enzyme (TACE). These substrates are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μM. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μl 1x HEPES buffer (0.2 M NaCl, 10 mM CaCl₂, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μl of substrate solution (50 μM) at 25 °C for 15 minutes, and then adding 20 μl of a purified fusion protein of the invention into the assay cuvette. The final concentration of substrate is 1 μM. Initial hydrolysis rates are monitored for 30-min.

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Example 60: Identification and Cloning of VH and VL domains

One method to identify and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent (Life Technologies, Rockville, MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75%

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ethanol. Following washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is then dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can be determined using optical density measurements.

5 cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transcriptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 3. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is
10 limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerase, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling
15 together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes are stored 4°C.

Table 3: Primer Sequences Used to Amplify VH and VL domains.

	Primer name	SEQ ID NO	Primer Sequence (5'-3')
	VH Primers		
5	Hu VH1-5'	36	CAGGTGCAGCTGGTGCAGTCTGG
	Hu VH2-5'	37	CAGGTCAACTTAAGGGAGTCTGG
	Hu VH3-5'	38	GAGGTGCAGCTGGTGGAGTCTGG
	Hu VH4-5'	39	CAGGTGCAGCTGCAGGAGTCGGG
	Hu VH5-5'	40	GAGGTGCAGCTGTTGCAGTCTGC
10	Hu VH6-5'	41	CAGGTACAGCTGCAGCAGTCAGG
	Hu JH1,2-5'	42	TGAGGAGACGGTGACCAGGGTGCC
	Hu JH3-5'	43	TGAAGAGACGGTGACCATTGTCCC
	Hu JH4,5-5'	44	TGAGGAGACGGTGACCAGGGTTCC
	Hu JH6-5'	45	TGAGGAGACGGTGACCGTGGTCCC
15	VL Primers		
	Hu Vkappa1-5'	46	GACATCCAGATGACCCAGTCTCC
	Hu Vkappa2a-5'	47	GATGTTGTGATGACTCAGTCTCC
	Hu Vkappa2b-5'	48	GATATTGTGATGACTCAGTCTCC
20	Hu Vkappa3-5'	49	GAAATTGTGTTGACGCAGTCTCC
	Hu Vkappa4-5'	50	GACATCGTGATGACCCAGTCTCC
	Hu Vkappa5-5'	51	GAAACGACACTCACGCAGTCTCC
	Hu Vkappa6-5'	52	GAAATTGTGCTGACTCAGTCTCC
	Hu Vlambda1-5'	53	CAGTCTGTGTTGACGCAGCCGCC
25	Hu Vlambda2-5'	54	CAGTCTGCCCTGACTCAGCCTGC
	Hu Vlambda3-5'	55	TCCTATGTGCTGACTCAGCCACC
	Hu Vlambda3b-5'	56	TCTTCTGAGCTGACTCAGGACCC
	Hu Vlambda4-5'	57	CACGTTATACTGACTCAACCGCC
	Hu Vlambda5-5'	58	CAGGCTGTGCTCACTCAGCCGTC
30	Hu Vlambda6-5'	59	AATTTTATGCTGACTCAGCCCCA
	Hu Jkappa1-3'	60	ACGTTTGATTTCCACCTTGGTCCC
	Hu Jkappa2-3'	61	ACGTTTGATCTCCAGCTTGGTCCC
	Hu Jkappa3-3'	62	ACGTTTGATATCCACTTTGGTCCC
	Hu Jkappa4-3'	63	ACGTTTGATCTCCACCTTGGTCCC
35	Hu Jkappa5-3'	64	ACGTTTAATCTCCAGTCGTGTCCC
	Hu Jlambda1-3'	65	CAGTCTGTGTTGACGCAGCCGCC
	Hu Jlambda2-3'	66	CAGTCTGCCCTGACTCAGCCTGC
	Hu Jlambda3-3'	67	TCCTATGTGCTGACTCAGCCACC
	Hu Jlambda3b-3'	68	TCTTCTGAGCTGACTCAGGACCC
40	Hu Jlambda4-3'	69	CACGTTATACTGACTCAACCGCC
	Hu Jlambda5-3'	70	CAGGCTGTGCTCACTCAGCCGTC
	Hu Jlambda6-3'	71	AATTTTATGCTGACTCAGCCCCA

PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc., Carlsbad, CA).

5 Individual cloned PCR products can be isolated after transfection of *E. coli* and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors
10 containing the nucleotide sequences of a heavy (e.g., human IgG1 or human IgG4) or light chain (human kappa or human lambda) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional antibody
15 molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domain to generate expression vectors that encode complete antibody molecules are well known within the art.

20 It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

25 The entire disclosure of each document cited (including patents, patent applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through Identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety. The specification and sequence listing of each of
30 the following U.S. applications are herein incorporated by reference in their entirety: Application Nos. 09/091,873 filed June 25, 1998; 60/229,358 filed on April 12, 2000; 60/199,384 filed on April 25, 2000; 60/256,931 filed on December 21, 2000, 09/809,269, filed March 16, 2001; 60/277,980, filed March 23, 2001; 09/236,557, filed January 26, 1999; 09/482,273, filed January 13, 2000; 60/234,925, filed November 1, 2000;
35 09/397,945, filed September 17, 1999; 09/296,622, filed April 23, 1999; 60/092,921, filed July 15, 1998; 09/305,736, filed May 5, 1999; 09/781,417, filed February 13, 2001; 60/152,317, filed September 3, 1999; 09/227,357, filed January 8, 1999; and 60/262,066,

filed January 18, 2001; and International Publication Nos. WO98/39446, filed September 11, 1998; WO 00/61625, filed October 19, 2000; WO/00/77022, filed December 21, 2000; and WO/00/76530, filed December 21, 2000.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit

11 April 2001

Accession Number

Unassigned

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*)

This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)


Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

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The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

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The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: Unassigned**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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Further deposits are identified on an additional sheet ☒

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Address of depositary institution (*including postal code and country*)

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United States of America

Date of deposit

11 April 2001

Accession Number

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Europe

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ATCC Deposit No.: Unassigned**CANADA**

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What is claimed:

1. An albumin fusion protein comprising a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:18.

5

2. An albumin fusion protein comprising a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity.

10

3. The albumin fusion protein of claim 2, wherein said albumin activity is the ability to prolong the shelf life of the Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused state.

15

4. The albumin fusion protein of claim 2, wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:18.

20

5. An albumin fusion protein comprising a fragment or variant of a Therapeutic protein:X, and albumin comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has a biological activity of the Therapeutic protein:X.

25

6. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin.

7. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin.

8. The albumin fusion protein of any one of claims 1-5, wherein the

Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin.

5 9. The albumin fusion protein of any one of claims 1-5, which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof.

10

 10. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker.

15

 11. The albumin fusion protein of any one of claims 1-8, wherein the albumin fusion protein has the following formula:

 R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

 wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment
20 or variant of albumin.

25

 12. The albumin fusion protein of any one of claims 1-11, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the Therapeutic protein:X in an unfused state.

 13. The albumin fusion protein of any one of claims 1-11, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

14. The albumin fusion protein of any one of claims 1-11, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

15. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

16. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising an amino acid sequence selected from the group consisting of:

- (a) amino acids 54 to 61 of SEQ ID NO:18;
- (b) amino acids 76 to 89 of SEQ ID NO:18;
- (c) amino acids 92 to 100 of SEQ ID NO:18;
- (d) amino acids 170 to 176 of SEQ ID NO:18;
- (e) amino acids 247 to 252 of SEQ ID NO:18;
- (f) amino acids 266 to 277 of SEQ ID NO:18;
- (g) amino acids 280 to 288 of SEQ ID NO:18;
- (h) amino acids 362 to 368 of SEQ ID NO:18;
- (i) amino acids 439 to 447 of SEQ ID NO:18;
- (j) amino acids 462 to 475 of SEQ ID NO:18;
- (k) amino acids 478 to 486 of SEQ ID NO:18; and
- (l) amino acids 560 to 566 of SEQ ID NO:18.

17. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life of the Therapeutic

protein:X , or a fragment or variant thereof, in an unfused state.

18. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

19. The albumin fusion protein of claims 15 or 16 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

20. The albumin fusion protein of any one of claims 1-19, which is non-glycosylated.

21. The albumin fusion protein of any one of claims 1-19, which is expressed in yeast.

22. The albumin fusion protein of claim 21, wherein the yeast is glycosylation deficient.

23. The albumin fusion protein of claim 21 wherein the yeast is glycosylation and protease deficient.

24. The albumin fusion protein of any one of claims 1-19, which is expressed by a mammalian cell.

25. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

26. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein further comprises a secretion leader sequence.

27. A composition comprising the albumin fusion protein of any one of claims 1-26 and a pharmaceutically acceptable carrier.

28. A kit comprising the composition of claim 27.

29. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-26.

30. The method of claim 29, wherein the disease or disorder comprises indication:Y.

31. A method of treating a patient with a disease or disorder that is modulated by Therapeutic protein:X, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 1-26.

32. The method of claim 31, wherein the disease or disorder is indication:Y.

33. A method of extending the shelf life of Therapeutic protein:X comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin or a fragment or variant thereof, sufficient to extend the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

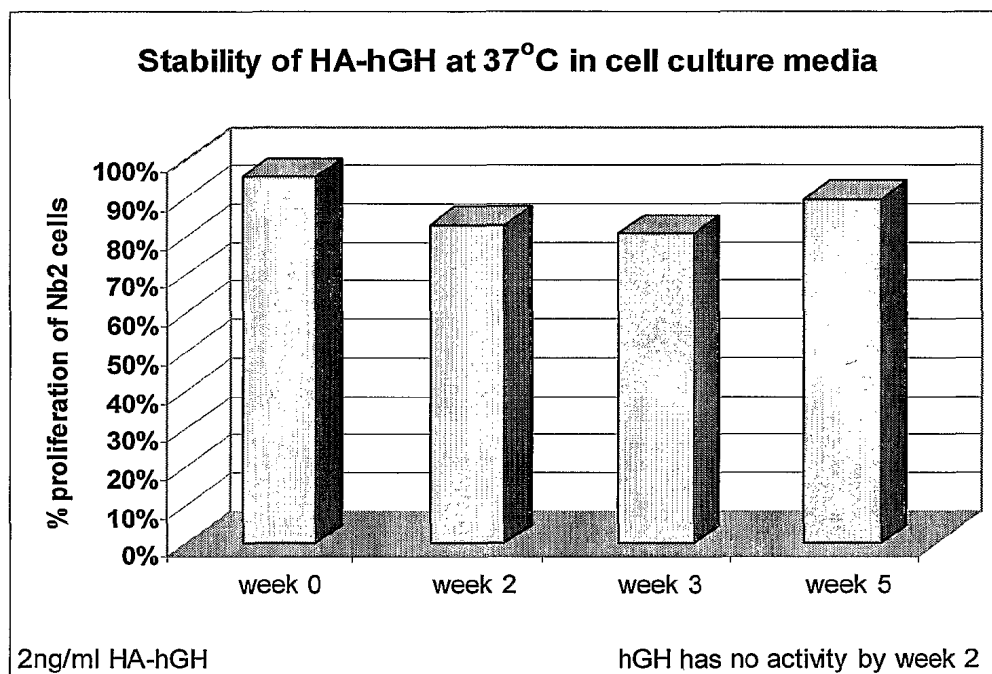
34. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-26.

35. A vector comprising the nucleic acid molecule of claim 34.

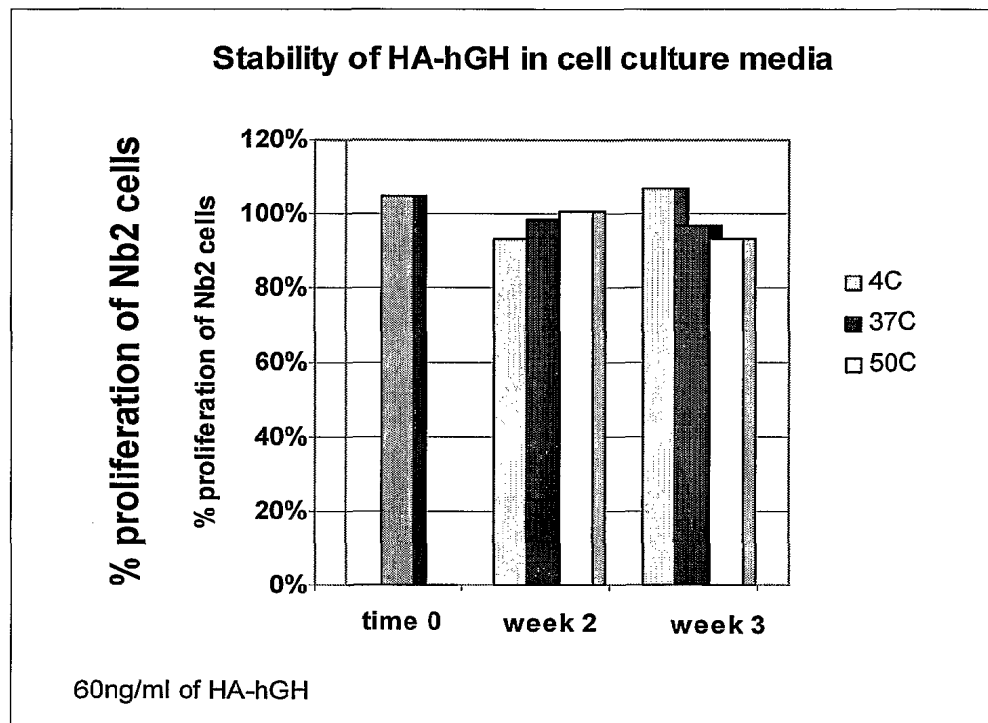
5

36. A host cell comprising the nucleic acid molecule of claim 35.

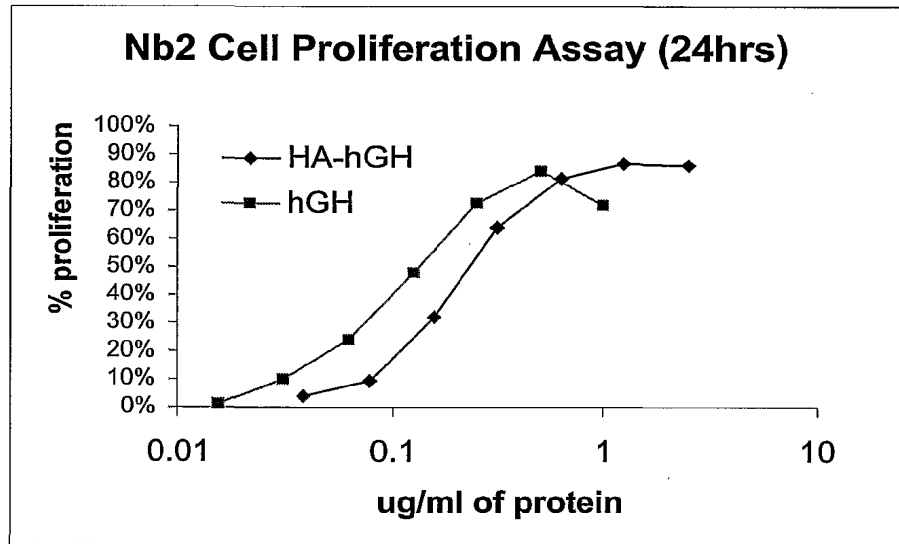
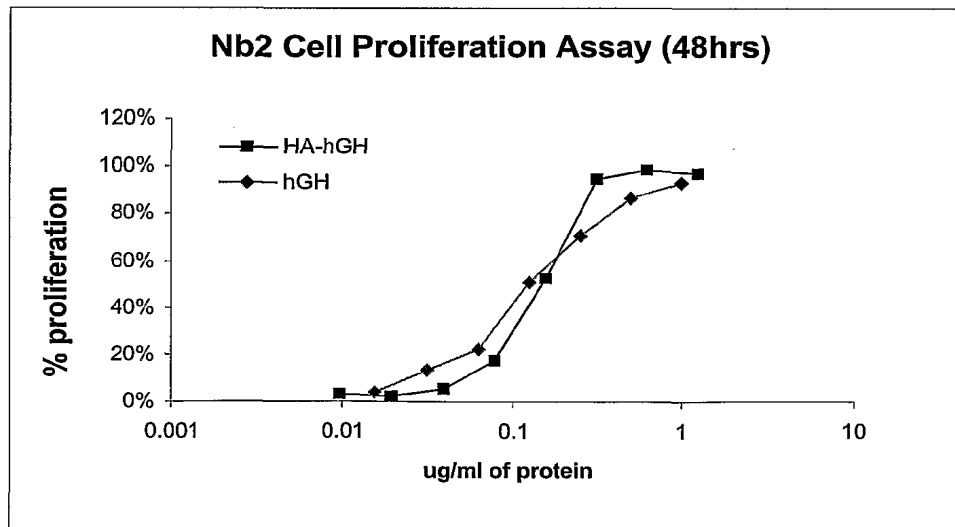
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**Figure 1**

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**Figure 2**

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**Figure 3A****Figure 3B**

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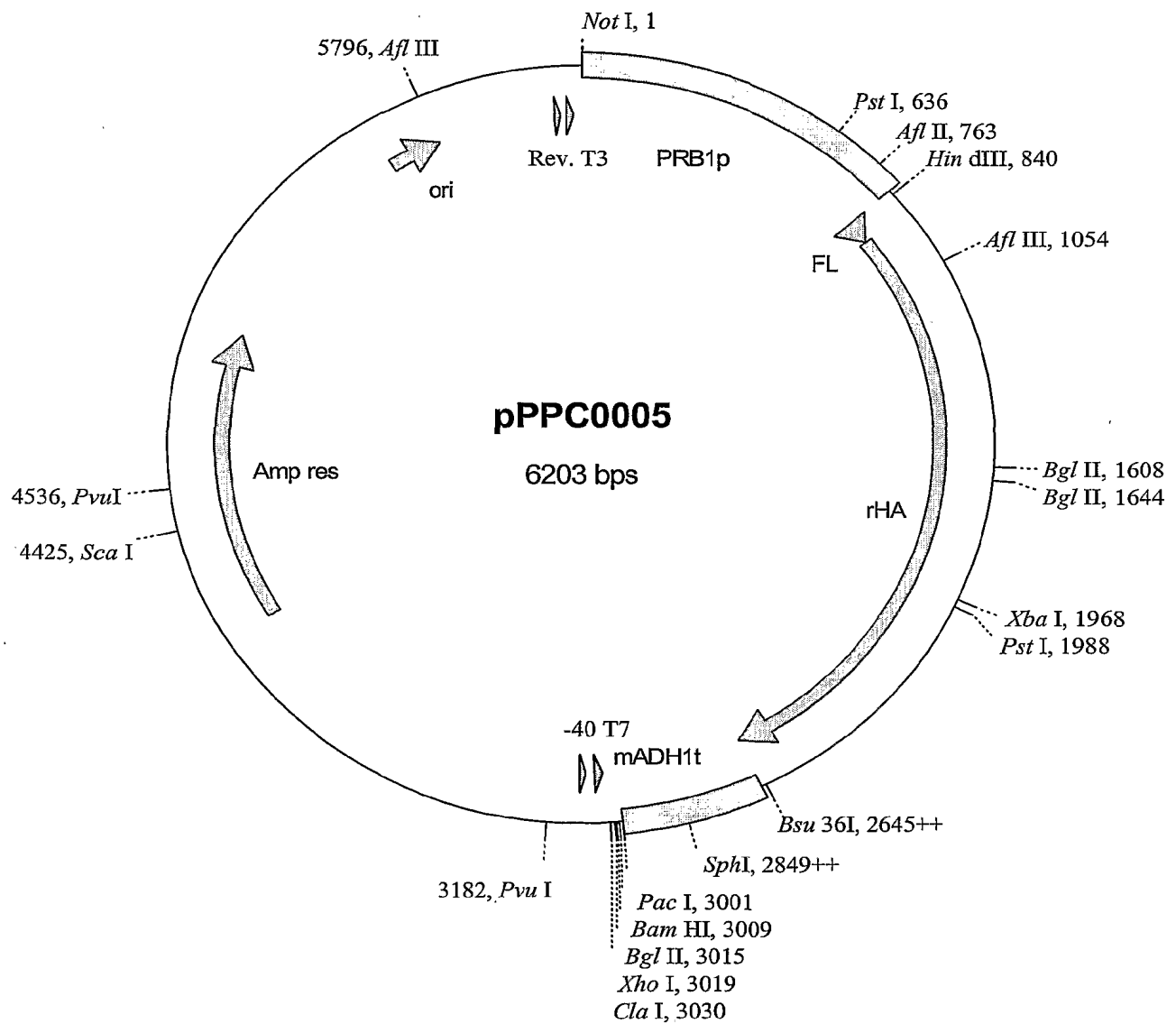
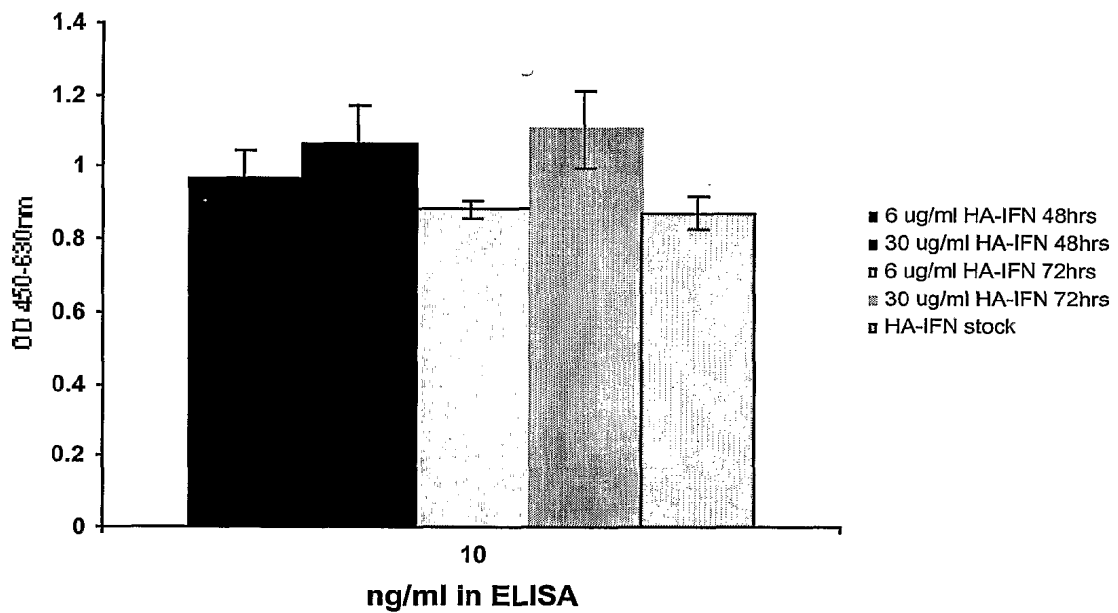


Figure 4

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**Figure 5**

6/20

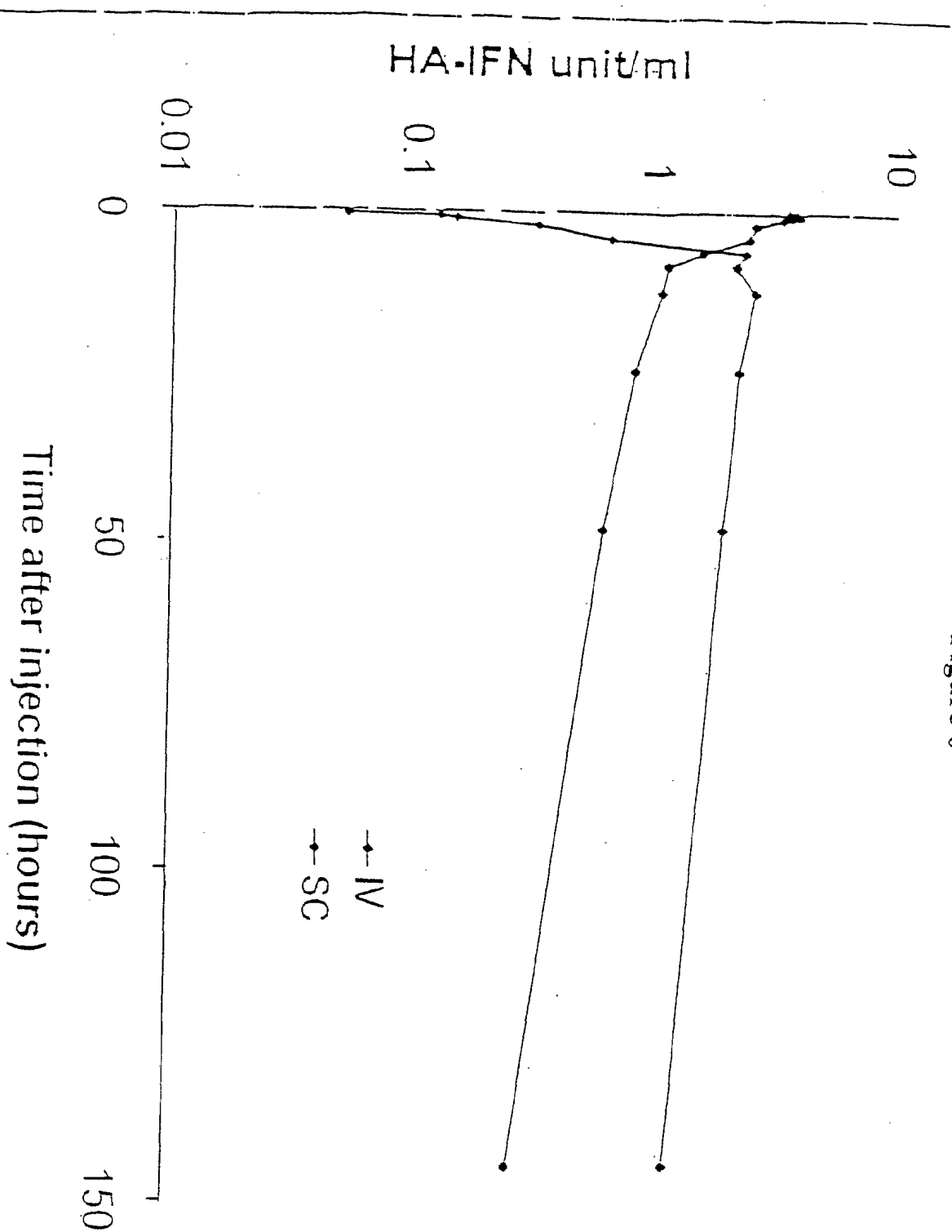


Figure 6

7/20

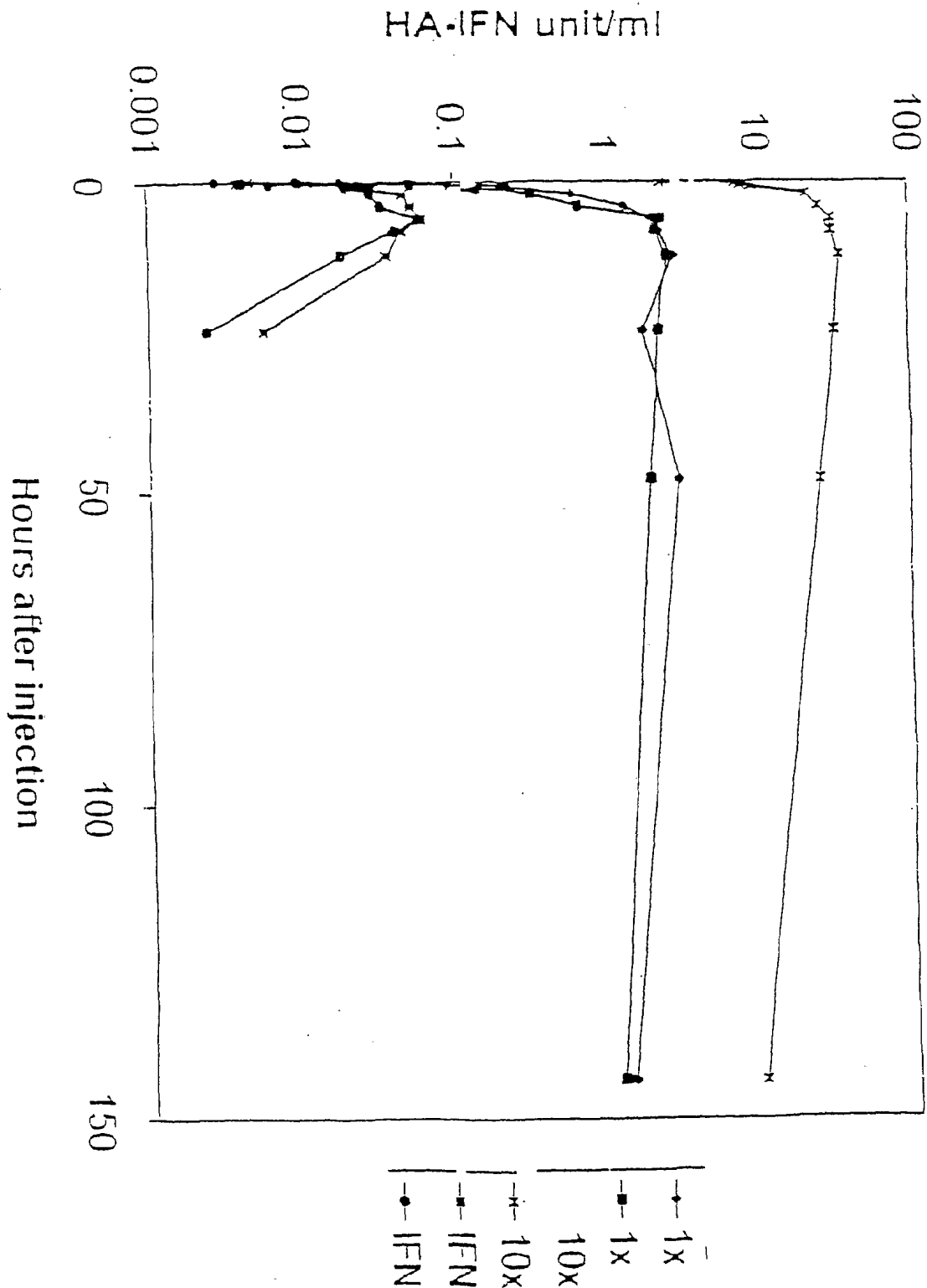


Figure 7

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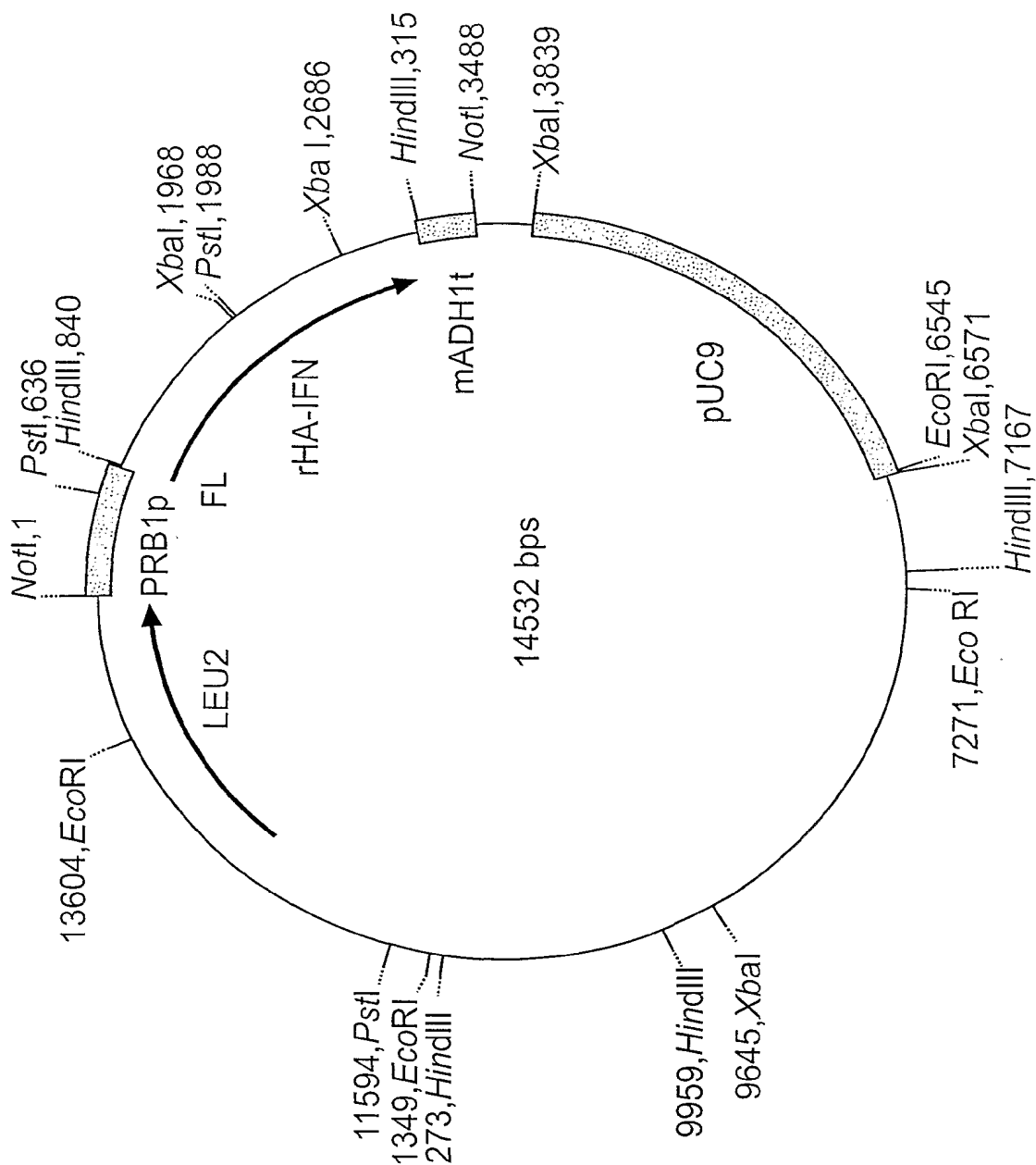


FIG. 8

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Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

1	DAHKSEVAHR	FKDLGEENFK	ALVLIAFAQY	LQQCPFEDHV	KLVNEVTEFA
	HHHHH	HHH	HHHHHHHHHH	HHHHH	HHHHHHHHHH
	I		II		III
51	KTCV ADESAE	NCDKSLHTLF	GDKLCT TVATL	RETYGEMADC	CAKOEPERNE
	HHHHH	HHHHH	HHHHH	HHHH	H HHHH
101	CFLQHKDDNP	NLPRLVRPEV	DVMCTAFHDN	EETFLKKYLY	EIARRHPYFY
	HHHH	H	HHHHHHHH	HHHHHHHHHH	HHHHH
	IV				
151	APELLFFAKR	YKAAFTECCO	AADKAA CLLP	KLDELRLDEGK	ASSAKQRLKC
	HHHHHHHHHH	HHHHHHHHH	HHHHH	HHHHHHHHHH	HHHHHHHHHH
	V				
201	ASLQKFGERA	FKAWAVARLS	QRFPKAEFAE	VSKLVTDLTK	VHTECC HGDL
	HHHHH	HH	HHHHHHHHHH	HH	HHH HHHHHHHHHH
	VI		VII		
251	LECADDRADL	AKYIC ENODS	ISSKLKECCE	KPLLEKSHCI	AEVENDEMPA
	HHHHHHHHHH	HHHHH	HHHHH	HHHHHHH	H
301	DLPSLAADFV	ESKDVCKNYA	EAKDVFLGMF	LYEYARRHPD	YSVVLRLRLA
	HHHH	HHHHHH	HHHHHHH	HHHHHH	HHHHHHHH
	VIII				
351	KTYETTTLEKC	CAAADPHECY	AKVFDEFKPL	VEEPQNLIKQ	NCELFEQLGE
	HHHHHHHHHH	HH	H	HHHHH	HHHHHHHHHH
	IX				
401	YKFQNALIVR	YTKKVPQVST	PTLVEVSRNL	GKVGSKCCKH	PEAKRMPCAE
	HHHHHHHHHH	HHHH	H	HHHHHHHHHH	HHH HHHHHHHH
	X		XI		
451	DYLSVVLNQL	CVLHEKTPVS	DRVTKCCTES	LVNRRPPCFSA	LEVDETYVPK
	HHHHHHHHHH	HHHHH	HHHHHHHHH	HHHHHHHH	
501	EFNAETFTFH	ADICTLSEKE	RQIKKQTALV	ELVKHKPKAT	KEQLKAVMDD
		HHH	HHH	HHHHMMEH	HHH HHHHHHHH
	XII				
551	FAAFVEKCKK	ADDKET CFAE	EGKKLVAASQ	AALGL	
	HHHHHHHH	HHHH	HHHHHHHHHH	HH	

Loop

I Val54-Asn61
 II Thr76-Asp89
 III Ala92-Glu100
 IV Gln170-Ala176
 V His247-Glu252
 VI Glu266-Glu277

Loop

VII Glu280-His288
 VIII Ala362-Glu368
 IX Lys439-Pro447
 X Val462-Lys475
 XI Thr478-Pro486
 XII Lys560-Thr566

Figure 9

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Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

```

151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHHH

```

IV

```

151  APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHHH

```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

(X)_n
↓
IV

```

151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHHH

```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

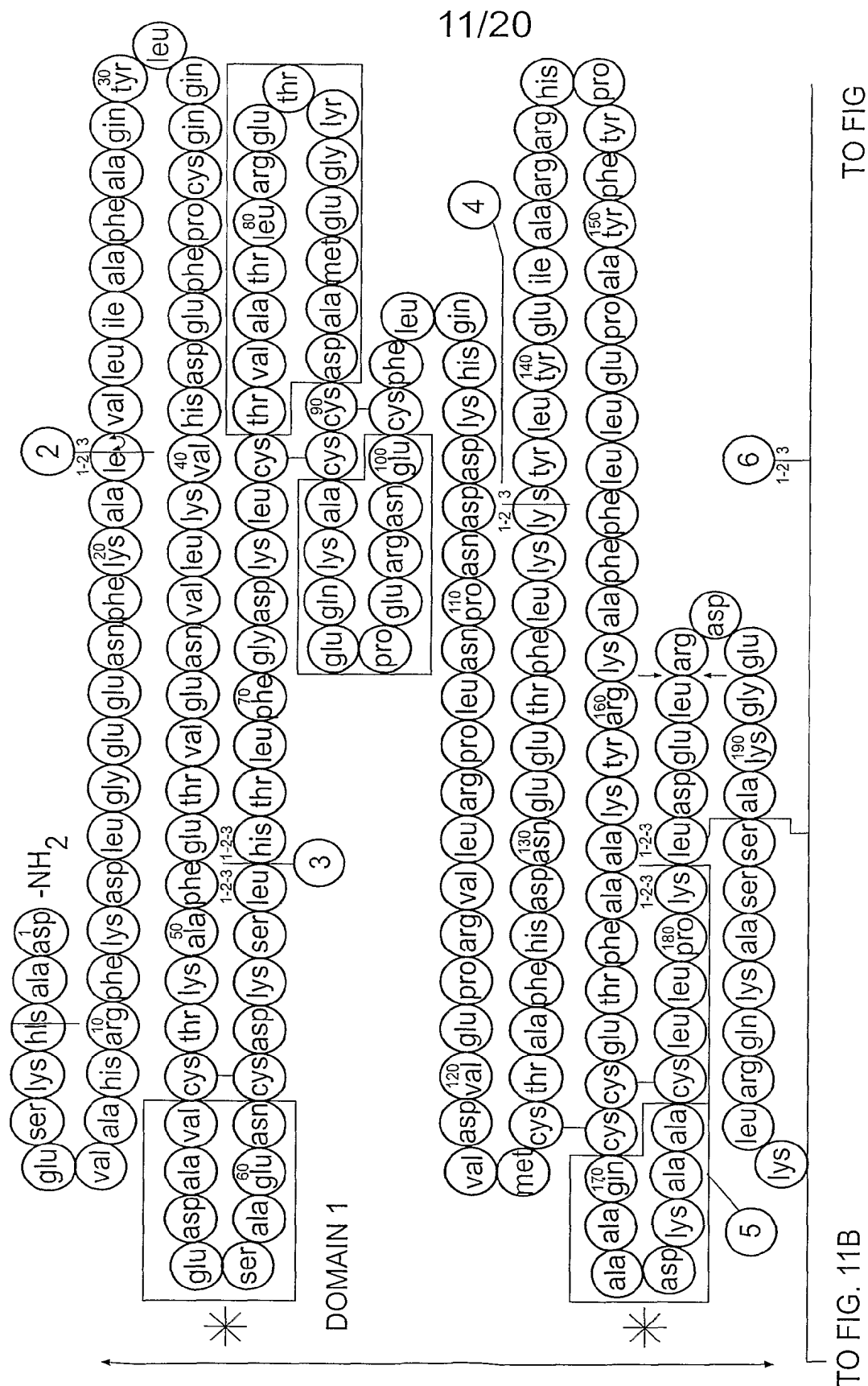
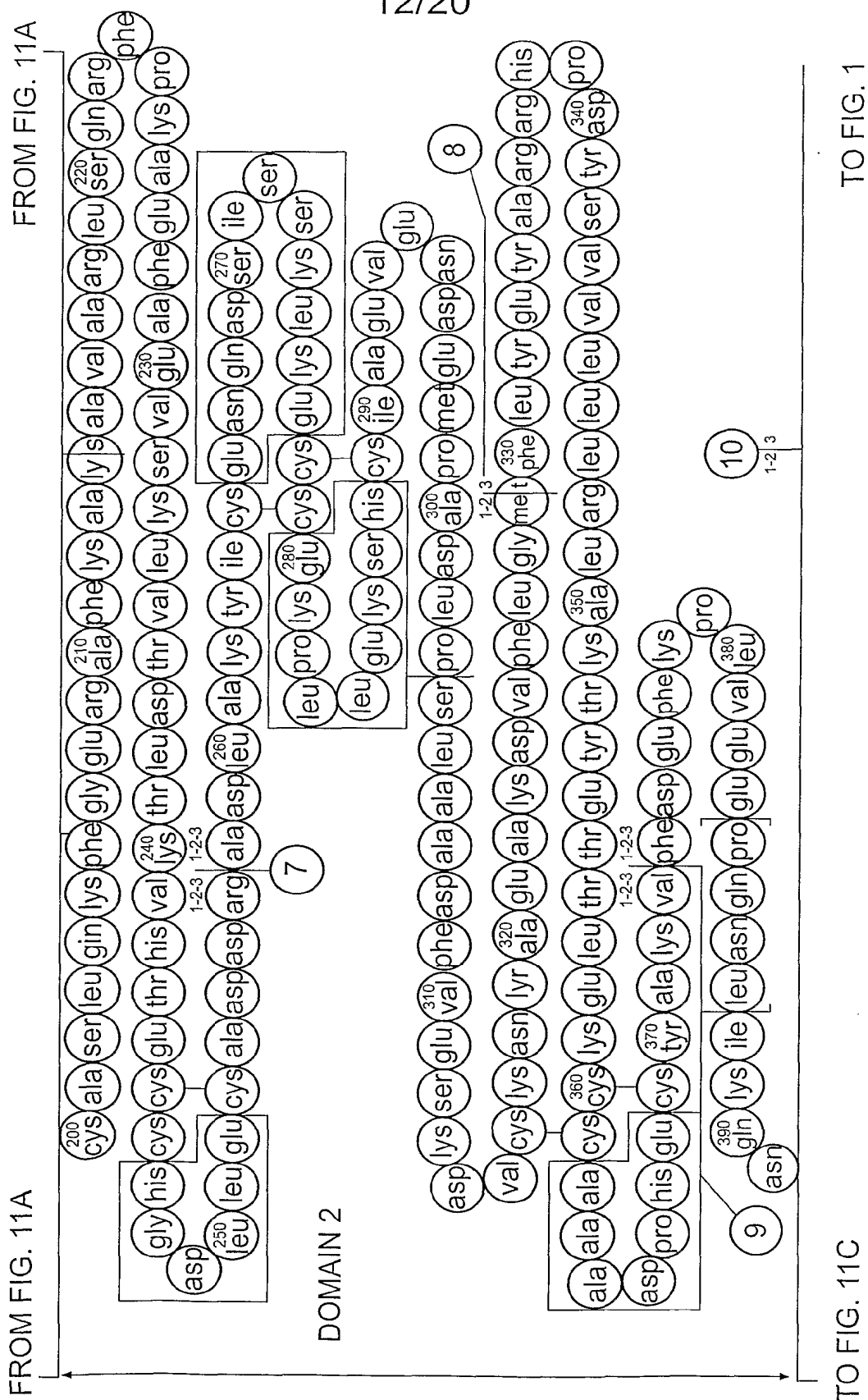


FIG. 11A

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TO FIG. 1

FIG. 11B

TO FIG. 11C

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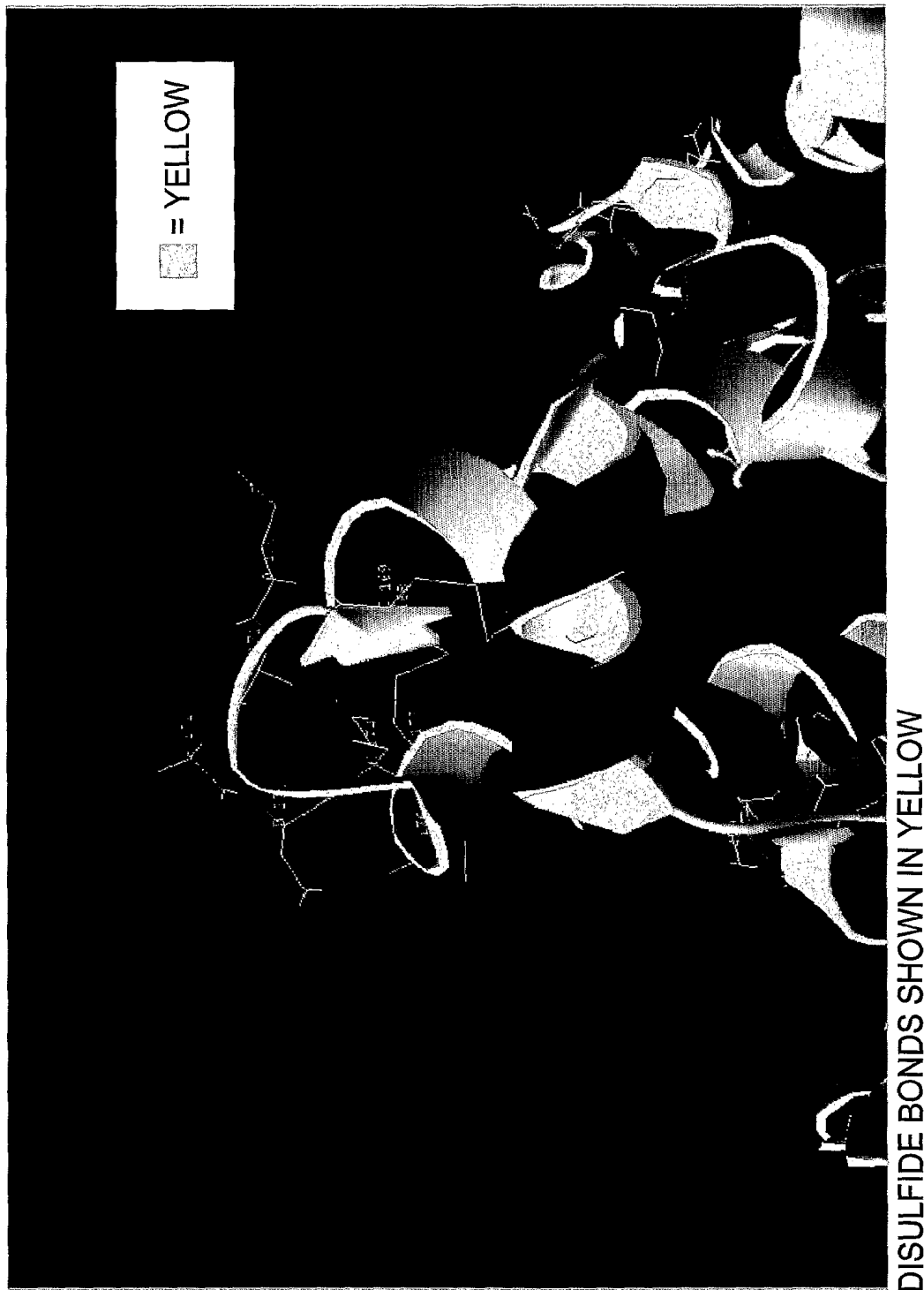


FIG. 12:
LOOP IV GLU170-A176

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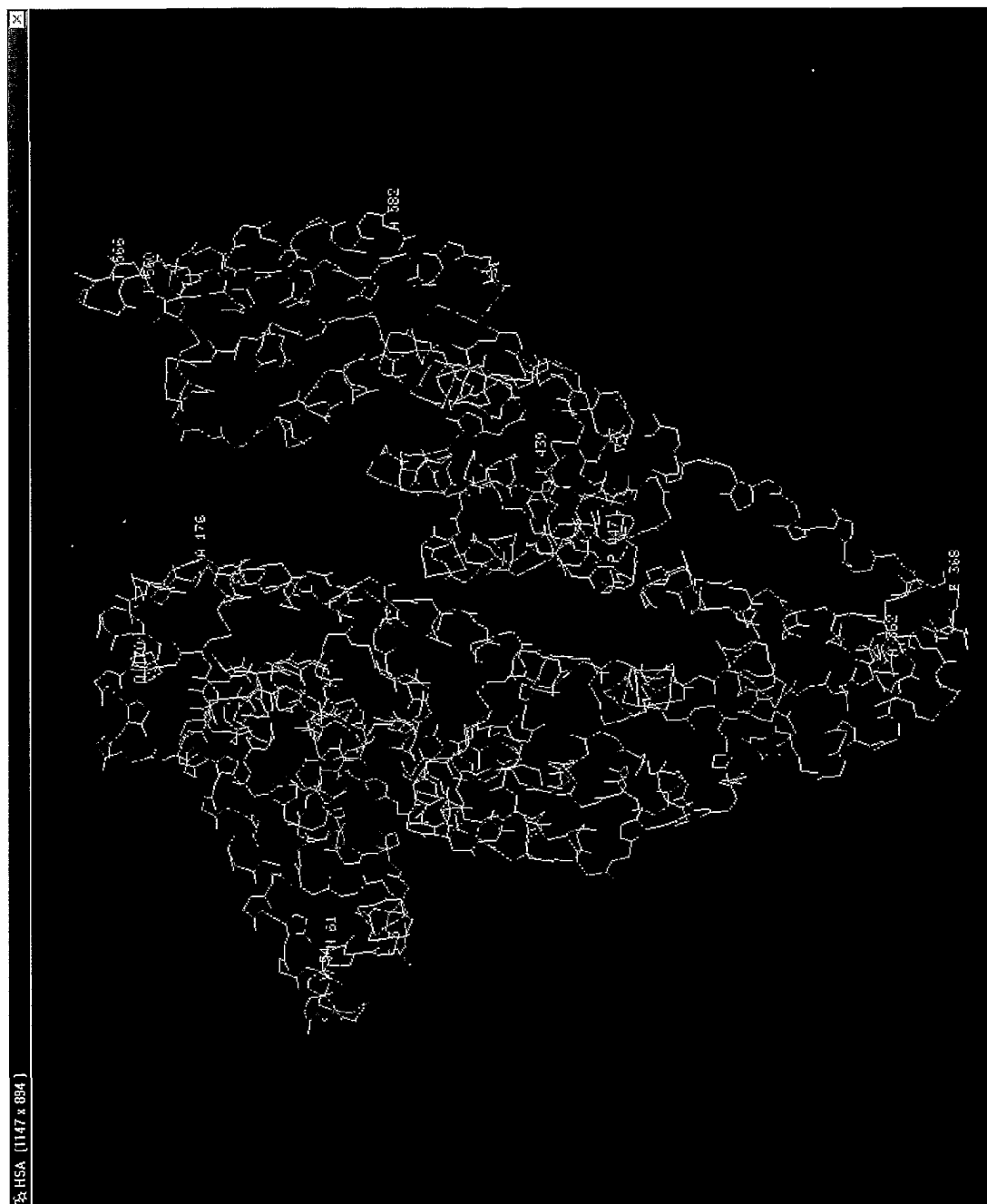


FIG. 13
TERTIARY STRUCTURE OF HA

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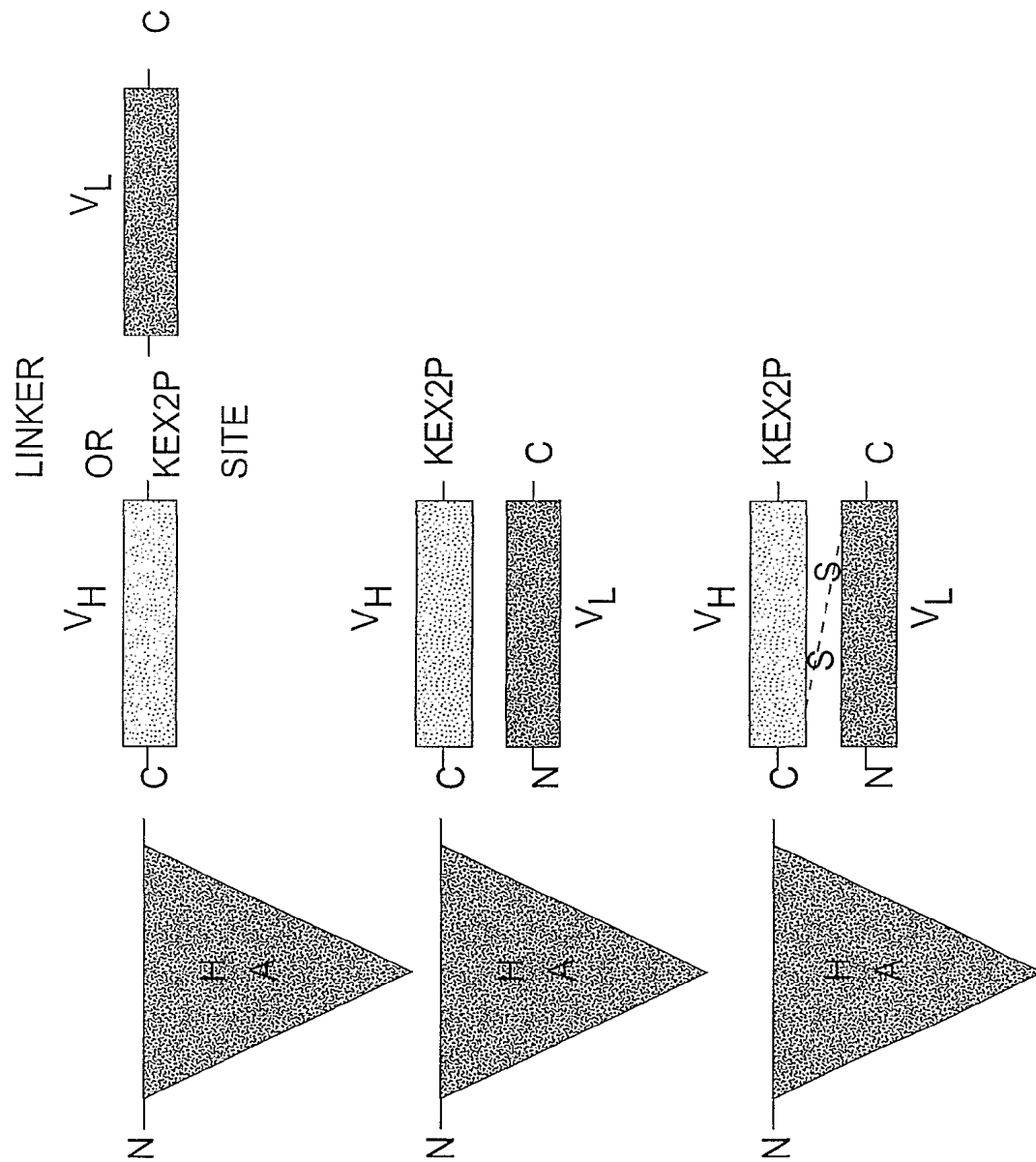


FIG. 14

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```

1  GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
1  D  A  H  K  S  E  V  A  H  R  F  K  D  L  G  E  E  N  F  K  20

61  GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21  A  L  V  L  I  A  F  A  Q  Y  L  Q  Q  C  P  F  E  D  H  V  40

121  AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
41  K  L  V  N  E  V  T  E  F  A  K  T  C  V  A  D  E  S  A  E  60

181  AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
61  N  C  D  K  S  L  H  T  L  F  G  D  K  L  C  T  V  A  T  L  80

241  CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
81  R  E  T  Y  G  E  M  A  D  C  C  A  K  Q  E  P  E  R  N  E  100

301  TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C  F  L  Q  H  K  D  D  N  P  N  L  P  R  L  V  R  P  E  V  120

361  GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D  V  M  C  T  A  F  H  D  N  E  E  T  F  L  K  K  Y  L  Y  140

421  GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E  I  A  R  R  H  P  Y  F  Y  A  P  E  L  L  F  F  A  K  R  160

```

Figure 15A

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```

481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D L P S L A A A D F V E S K D V C K N Y A 320

```

Figure 15B

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```

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
341 Y S V V L L L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
361 C A A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
461 C V L H E K T P V S D R V T K C C T E S 480

```

Figure 15C

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```

1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
481 L V N R R R P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
501 E F N A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
541 K E Q L K A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
581 A A L G L * 585

```

Figure 15D

SEQUENCE LISTING

<110> Human Genome Sciences, Inc.

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<130> PF545PCT

<140> Unassigned

<141> 2001-04-12

<150> 60/229,358

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<151> 2000-04-25

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<210> 1

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<400> 1

cccaagaatt cccttatcca ggc

23

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<211> 33

<212> DNA

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<223> primer useful to clone human growth hormone cDNA

<400> 2

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33

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<212> DNA

<213> Artificial Sequence

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with non-cohesive ends.

<400> 3
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<210> 4
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<400> 4
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<210> 5
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<212> DNA
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with non-cohesive ends.

<400> 5
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<210> 6
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<221> misc_structure
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with non-cohesive ends.

<400> 6
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<210> 7
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<213> Artificial Sequence

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<222> 1)..(19)
<223> invertase leader sequence

<220>
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<222> 20)..(24)

<223> first 5 amino acids of mature human serum albumin

<400> 7

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 1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser
 20

<210> 8

<211> 21

<212> DNA

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21

<210> 9

<211> 27

<212> DNA

<213> Artificial Sequence

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27

<210> 10

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<400> 10

ctcttggtgtg catcgaagcc acag

24

<210> 11

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> synthetic oligonucleotide used to join DNA fragments with non-cohesive ends.

<400> 11

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30

<210> 12

<211> 31

<212> DNA

<213> Artificial Sequence

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<400> 12

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31

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<400> 13

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47

<210> 14

<211> 48

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<213> Artificial Sequence

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<400> 14

aattggtggg aaagatccac caccgccgga tccaccgcca cctaagcc

48

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<211> 62

<212> DNA

<213> Artificial Sequence

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<400> 15

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ac 62

<210> 16

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_structure

<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 16

aattgttggg aaggatccac cgccaccaga tccgccgcca ccagatccac caccgcctaa 60
gcc 63

<210> 17

<211> 1782

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1755)

<400> 17

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1 5 10 15

gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag 96
Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
20 25 30

cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa 144
Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35 40 45

ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50 55 60

tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt 240
Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65 70 75 80

cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85 90 95

gag aga aat gaa tgc ttc ttg caa cac aaa gat gac aac cca aac ctc 336
Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

ccc cga ttg gtg aga cca gag gtt gat gtg atg tgc act gct ttt cat 384
Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His

115	120	125	
gac aat gaa gag aca ttt ttg	aaa aaa tac tta tat gaa att gcc aga	432	
Asp Asn Glu Glu Thr Phe Leu	Lys Lys Tyr Leu Tyr Glu Ile Ala Arg		
130	135 140		
aga cat cct tac ttt tat gcc ccg	gaa ctc ctt ttc ttt gct aaa agg	480	
Arg His Pro Tyr Phe Tyr	Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg		
145	150 155 160		
tat aaa gct gct ttt aca gaa tgt tgc	caa gct gct gat aaa gct gcc	528	
Tyr Lys Ala Ala Phe Thr Glu Cys Cys	Gln Ala Ala Asp Lys Ala Ala		
	165 170 175		
tgc ctg ttg cca aag ctc gat gaa ctt	cggtgat gaa ggg aag gct tcg	576	
Cys Leu Leu Pro Lys Leu Asp Glu	Leu Arg Asp Glu Gly Lys Ala Ser		
	180 185 190		
tct gcc aaa cag aga ctc aaa tgt gcc	agt ctc caa aaa ttt gga gaa	624	
Ser Ala Lys Gln Arg Leu Lys Cys	Ala Ser Leu Gln Lys Phe Gly Glu		
	195 200 205		
aga gct ttc aaa gca tgg gca gtg gct	cgctctg agc cag aga ttt ccc	672	
Arg Ala Phe Lys Ala Trp Ala Val	Ala Arg Leu Ser Gln Arg Phe Pro		
	210 215 220		
aaa gct gag ttt gca gaa gtt tcc aag	tta gtg aca gat ctt acc aaa	720	
Lys Ala Glu Phe Ala Glu Val Ser	Lys Leu Val Thr Asp Leu Thr Lys		
	225 230 235 240		
gtc cac acg gaa tgc tgc cat gga gat	ctg ctt gaa tgt gct gat gac	768	
Val His Thr Glu Cys Cys His Gly	Asp Leu Leu Glu Cys Ala Asp Asp		
	245 250 255		
agg gcg gac ctt gcc aag tat atc tgt	gaa aat cag gat tcg atc tcc	816	
Arg Ala Asp Leu Ala Lys Tyr Ile	Cys Glu Asn Gln Asp Ser Ile Ser		
	260 265 270		
agt aaa ctg aag gaa tgc tgt gaa aaa	cct ctg ttg gaa aaa tcc cac	864	
Ser Lys Leu Lys Glu Cys Cys Glu	Lys Pro Leu Leu Glu Lys Ser His		
	275 280 285		
tgc att gcc gaa gtg gaa aat gat gag	atg cct gct gac ttg cct tca	912	
Cys Ile Ala Glu Val Glu Asn Asp	Glu Met Pro Ala Asp Leu Pro Ser		
	290 295 300		
tta gct gct gat ttt gtt gaa agt aag	gat gtt tgc aaa aac tat gct	960	
Leu Ala Ala Asp Phe Val Glu Ser	Lys Asp Val Cys Lys Asn Tyr Ala		
	305 310 315 320		
gag gca aag gat gtc ttc ctg ggc atg	ttt ttg tat gaa tat gca aga	1008	
Glu Ala Lys Asp Val Phe Leu Gly	Met Phe Leu Tyr Glu Tyr Ala Arg		
	325 330 335		
agg cat cct gat tac tct gtc gtg ctg	ctg ctg ctg aga ctt gcc aag aca	1056	
Arg His Pro Asp Tyr Ser Val Val	Leu Leu Leu Arg Leu Ala Lys Thr		
	340 345 350		
tat gaa acc act cta gag aag tgc tgt	gcc gct gca gat cct cat gaa	1104	
Tyr Glu Thr Thr Leu Glu Lys Cys	Cys Ala Ala Ala Asp Pro His Glu		

355				360				365								
tgc	tat	gcc	aaa	gtg	ttc	gat	gaa	ttt	aaa	cct	ctt	gtg	gaa	gag	cct	1152
Cys	Tyr	Ala	Lys	Val	Phe	Asp	Glu	Phe	Lys	Pro	Leu	Val	Glu	Glu	Pro	
	370					375					380					
cag	aat	tta	atc	aaa	caa	aac	tgt	gag	ctt	ttt	gag	cag	ctt	gga	gag	1200
Gln	Asn	Leu	Ile	Lys	Gln	Asn	Cys	Glu	Leu	Phe	Glu	Gln	Leu	Gly	Glu	
385					390					395					400	
tac	aaa	ttc	cag	aat	gcg	cta	tta	gtt	cgt	tac	acc	aag	aaa	gta	ccc	1248
Tyr	Lys	Phe	Gln	Asn	Ala	Leu	Leu	Val	Arg	Tyr	Thr	Lys	Lys	Val	Pro	
				405					410					415		
caa	gtg	tca	act	cca	act	ctt	gta	gag	gtc	tca	aga	aac	cta	gga	aaa	1296
Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Glu	Val	Ser	Arg	Asn	Leu	Gly	Lys	
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gtg	ggc	agc	aaa	tgt	tgt	aaa	cat	cct	gaa	gca	aaa	aga	atg	ccc	tgt	1344
Val	Gly	Ser	Lys	Cys	Cys	Lys	His	Pro	Glu	Ala	Lys	Arg	Met	Pro	Cys	
	435					440						445				
gca	gaa	gac	tat	cta	tcc	gtg	gtc	ctg	aac	cag	tta	tgt	gtg	ttg	cat	1392
Ala	Glu	Asp	Tyr	Leu	Ser	Val	Val	Leu	Asn	Gln	Leu	Cys	Val	Leu	His	
	450					455				460						
gag	aaa	acg	cca	gta	agt	gac	aga	gtc	aca	aaa	tgc	tgc	aca	gag	tcc	1440
Glu	Lys	Thr	Pro	Val	Ser	Asp	Arg	Val	Thr	Lys	Cys	Cys	Thr	Glu	Ser	
465					470				475						480	
ttg	gtg	aac	agg	cga	cca	tgc	ttt	tca	gct	ctg	gaa	gtc	gat	gaa	aca	1488
Leu	Val	Asn	Arg	Arg	Pro	Cys	Phe	Ser	Ala	Leu	Glu	Val	Asp	Glu	Thr	
				485					490					495		
tac	gtt	ccc	aaa	gag	ttt	aat	gct	gaa	aca	ttc	acc	ttc	cat	gca	gat	1536
Tyr	Val	Pro	Lys	Glu	Phe	Asn	Ala	Glu	Thr	Phe	Thr	Phe	His	Ala	Asp	
			500					505					510			
ata	tgc	aca	ctt	tct	gag	aag	gag	aga	caa	atc	aag	aaa	caa	act	gca	1584
Ile	Cys	Thr	Leu	Ser	Glu	Lys	Glu	Arg	Gln	Ile	Lys	Lys	Gln	Thr	Ala	
		515					520					525				
ctt	gtt	gag	ctt	gtg	aaa	cac	aag	ccc	aag	gca	aca	aaa	gag	caa	ctg	1632
Leu	Val	Glu	Leu	Val	Lys	His	Lys	Pro	Lys	Ala	Thr	Lys	Glu	Gln	Leu	
	530					535					540					
aaa	gct	gtt	atg	gat	gat	ttc	gca	gct	ttt	gta	gag	aag	tgc	tgc	aag	1680
Lys	Ala	Val	Met	Asp	Asp	Phe	Ala	Ala	Phe	Val	Glu	Lys	Cys	Cys	Lys	
545					550				555						560	
gct	gac	gat	aag	gag	acc	tgc	ttt	gcc	gag	gag	ggg	aaa	aaa	ctt	gtt	1728
Ala	Asp	Asp	Lys	Glu	Thr	Cys	Phe	Ala	Glu	Glu	Gly	Lys	Lys	Leu	Val	
				565				570						575		
gct	gca	agt	caa	gct	gcc	tta	ggc	tta	taacatctac attttaaagc atctcag							1782
Ala	Ala	Ser	Gln	Ala	Ala	Leu	Gly	Leu								
			580					585								

<211> 585

<212> PRT

<213> Homo Sapiens

<400> 18

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Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
          20              25              30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
          35              40              45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
 50              55              60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65              70              75              80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
          85              90              95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100              105              110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
115              120              125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
130              135              140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145              150              155              160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
          165              170              175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
          180              185              190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
195              200              205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
210              215              220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225              230              235              240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
          245              250              255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
260              265              270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
275              280              285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser

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290	295	300
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala 305 310 315 320		
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Arg His Pro Asp Tyr Ser Val Val Leu Leu Arg Leu Ala Lys Thr 340 345 350		
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu 355 360 365		
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro 370 375 380		
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu 385 390 395 400		
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro 405 410 415		
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys 420 425 430		
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys 435 440 445		
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His 450 455 460		
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser 465 470 475 480		
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr 485 490 495		
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp 500 505 510		
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala 515 520 525		
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu 530 535 540		
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys 545 550 555 560		
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<211> 60

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<221> Misc_Structure

<223> Synthetic oligonucleotide used to alter restriction sites in pPPC0007

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33

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1             5             10             15

Tyr Ser Arg Ser Leu Asp Lys Arg
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<210> 30
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albumin fusion VECTOR

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tcagggatcc aagcttccgc caccatgaag tgggtaacct ttatttcctc tctttttctc 60

tttagctcgg cttactcgag ggggtgtgttt cgctcgagatg cacacaagag tgag 114

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<223> Asp718 restriction site

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gcagcggtac cgaattcggc gcgccttata agcctaaggc agc 43

<210> 32
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46

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<210> 34
 <211> 17
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 1 5 10 15

Ala

<210> 35
 <211> 22
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 1 5 10 15

Trp Ala Pro Ala Arg Gly
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<210> 36
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caggtgcagc tgggtgcagtc tgg

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<211> 23

<212> DNA

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<210> 38

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<212> DNA

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<210> 42
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<210> 43
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<400> 43
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<210> 44
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<210> 45
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<210> 46

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<212> DNA

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<223>Degenerate Vkappa forward primer useful for
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<210> 47

<211> 23

<212> DNA

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<212> DNA

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<210> 50
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<210> 51
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<210> 54
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<400> 55
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 145 150 155 160
 Pro Thr Ser Cys Ser Arg Cys
 165

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution (including postal code and country)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit

11 April 2001

Accession Number

PTA-3276

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

For receiving Office use only			For International Bureau use only		
<input type="checkbox"/> This sheet was received with the international application			<input type="checkbox"/> This sheet was received by the International Bureau on		
Authorized officer			Authorized officer		

ATCC Deposit No.: PTA-3276

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-3276

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution (including postal code and country)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit

11 April 2001

Accession Number

PTA-3277

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")

	For receiving Office use only			For International Bureau use only	
<input type="checkbox"/> This sheet was received with the international application			<input type="checkbox"/> This sheet was received by the International Bureau on		
Authorized officer			Authorized officer		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/11941

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 37/02; C12N 15/00

US CL : 530/350; 435/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
EST, GENEMBL, AGENESEQ

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE, BIOSIS, USPAT, JAPIO, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/23857 A1 (DELTA BIOTECHNOLOGY LIMITED) 08 September 1995 (08.09.95), see entire document.	1-9, 15-19
X	EP 0 322 094 A1 (DELTA BIOTECHNOLOGY LIMITED) 28 June 1989 (28.06.89), see entire document.	1-9, 15-19



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

18 JULY 2001

Date of mailing of the international search report

14 AUG 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

HOPE ROBINSON

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/11991

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 10-14, 20-32 and 34-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 15-19 (FGF-7)

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Groups 1-58, claim(s) 1-9, 15-19, all in part, drawn to a therapeutic protein X, wherein X correlates to those listed in the Table on page 11 of the description. If any of Groups 1-58 are elected the claims will only be examined in-so-far-as it pertains to the elected protein X. For example,

If Group 1 is elected, this correlates to Therapeutic Protein X: FGF-7.

Group 2, all partially as in Group 1, concerning Therapeutic Protein X: Kertainocyte growth factor 2.

Group 3, all partially as in Group 1, concerning Therapeutic Protein X: C-C Chemokine Receptor 5.

Group 4, all partially as in Group 1, concerning Therapeutic Protein X: Cathepsin K.

Group 5, all partially as in Group 1, concerning Therapeutic Protein X: MPIF-1 (Myeloid Progenitor Inhibitory Factor).

Group 6, all partially as in Group 1, concerning Therapeutic Protein X: VEGF.

Group 7, all partially as in Group 1, concerning Therapeutic Protein X: VEGF-2.

Group 8, all partially as in Group 1, concerning Therapeutic Protein X: BLyS.

Group 9, all partially as in Group 1, concerning Therapeutic Protein X: KD1.

Group 10, all partially as in Group 1, concerning Therapeutic Protein X: TMP-1.

Group 11, all partially as in Group 1, concerning Therapeutic Protein X: TMP-2.

Group 12, all partially as in Group 1, concerning Therapeutic Protein X: TMP-3.

Group 13, all partially as in Group 1, concerning Therapeutic Protein X: TMP-4.

Group 14, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor Protein.

Group 15, all partially as in Group 1, concerning Therapeutic Protein X: CTGF-2.

Group 16, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor 4.

Group 17, all partially as in Group 1, concerning Therapeutic Protein X: Human T-cell Lymphoma-lipoprotein associated phospholipase-A2.

Group 18, all partially as in Group 1, concerning Therapeutic Protein X: VEG1.

Group 19, all partially as in Group 1, concerning Therapeutic Protein X: AIM-1.

Group 20, all partially as in Group 1, concerning Therapeutic Protein X: TNF-delta.

Group 21, all partially as in Group 1, concerning Therapeutic Protein X: TNF-epsilon.

Group 22, all partially as in Group 1, concerning Therapeutic Protein X: AIM-2.

Group 23, all partially as in Group 1, concerning Therapeutic Protein X: Endokine.

Group 24, all partially as in Group 1, concerning Therapeutic Protein X: TR1.

Group 25, all partially as in Group 1, concerning Therapeutic Protein X: TR2.

Group 26, all partially as in Group 1, concerning Therapeutic Protein X: DR3.

Group 27, all partially as in Group 1, concerning Therapeutic Protein X: TR4.

Group 28, all partially as in Group 1, concerning Therapeutic Protein X: 4-1BBSv receptor.

Group 29, all partially as in Group 1, concerning Therapeutic Protein X: OPGL.

Group 30, all partially as in Group 1, concerning Therapeutic Protein X: FasL.

Group 31, all partially as in Group 1, concerning Therapeutic Protein X: Fas.

Group 32, all partially as in Group 1, concerning Therapeutic Protein X: TR5.

Group 33, all partially as in Group 1, concerning Therapeutic Protein X: TR6.

Group 34, all partially as in Group 1, concerning Therapeutic Protein X: DR5.

Group 35, all partially as in Group 1, concerning Therapeutic Protein X: TR8.

Group 36, all partially as in Group 1, concerning Therapeutic Protein X: TR9.

Group 37, all partially as in Group 1, concerning Therapeutic Protein X: TR10.

Group 38, all partially as in Group 1, concerning Therapeutic Protein X: TR11.

Group 39, all partially as in Group 1, concerning Therapeutic Protein X: TR12.

Group 40, all partially as in Group 1, concerning Therapeutic Protein X: TR13.

Group 41, all partially as in Group 1, concerning Therapeutic Protein X: TR14.

Group 42, all partially as in Group 1, concerning Therapeutic Protein X: TR16.

Group 43, all partially as in Group 1, concerning Therapeutic Protein X: HLD0U18.

Group 44, all partially as in Group 1, concerning Therapeutic Protein X: HSDSB09.

Group 45, all partially as in Group 1, concerning Therapeutic Protein X: HDPBQ71.

Group 46, all partially as in Group 1, concerning Therapeutic Protein X: HAGDG59.

Group 47, all partially as in Group 1, concerning Therapeutic Protein X: HCHNF25.

Group 48, all partially as in Group 1, concerning Therapeutic Protein X: HKACD58.

Group 49, all partially as in Group 1, concerning Therapeutic Protein X: HWACB86.

Group 50, all partially as in Group 1, concerning Therapeutic Protein X: HFTCF50.

Group 51, all partially as in Group 1, concerning Therapeutic Protein X: HRDFD27.

Group 52, all partially as in Group 1, concerning Therapeutic Protein X: HCEGG08.

Group 53, all partially as in Group 1, concerning Therapeutic Protein X: HKACI79.

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Group 54, all partially as in Group 1, concerning Therapeutic Protein X: HWHGZ51.
Group 55, all partially as in Group 1, concerning Therapeutic Protein X: HDTAI21.
Group 56, all partially as in Group 1, concerning Therapeutic Protein X: HCNCA73.
Group 57, all partially as in Group 1, concerning Therapeutic Protein X: HNHFE71.
Group 58, all partially as in Group 1, concerning Therapeutic Protein X: HLWCF05.

Group 59-117, claim(s) 33, drawn to a method of extending shelf life of Therapeutic Protein X, wherein Therapeutic Protein X can be any of the proteins listed in the Table on page 11 of the description. If any of Groups 59-117 are elected the claim will only be examined in-so-far-as it pertains to the elected Protein X.

The inventions listed as Groups 1-118 do not relate to a single inventive concept under PCT Rule 18.1 because, under PCT Rule 18.2, they lack the same or corresponding special technical features for the following reasons: because the technical feature linking groups 1-118 is not special because Inventions 1-58 do not avoid the prior art as Delta Biotechnology Limited, (EP 322094, June 28, 1989) teaches the claimed sequence. Thus, the invention does not relate to a single inventive concept and is not a contribution over the prior art.